



UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of : Linda H. Malkas
By the Examiner : Huff, Sheela Jitendra
Docket No. : 80371/5
Serial No. : 10/083,576
Filed : February 27, 2002
Group Art Unit : 1643
Title : METHOD FOR PURIFYING CANCER-SPECIFIC
PROLIFERATING CELL NUCLEAR ANTIGEN

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.132

Dear Sir:

I, Pamela E. Bechtel, declare as follows:

1. I am a co-inventor, along with Linda H. Malkas, Robert J. Hickey, Lauren Schnaper, Min Park, Derek J. Hoelz, and Dragana Tomic, of the subject matter disclosed and claimed in the United States Patent Application Serial No. 10/083,576, filed February 27, 2002, entitled METHOD FOR PURIFYING CANCER SPECIFIC PROLIFERATING CELL NUCLEAR ANTIGEN.

2. I am a co-author of an article entitled *An Altered Form of Proliferating Cell Nuclear Antigen in Various Cancers* (EXHIBIT A), that was included as part of the Doctoral Dissertation of Pamela E. Bechtel entitled *Proliferating Cell Nuclear Antigen in Malignancy*, that was submitted to the Faculty of the Graduate School of the University of Maryland in 1998 and approved May 8, 1998, and am a co-inventor of the subject matter which is disclosed in this publication and disclosed and claimed in the above-referenced patent application.

3. In addition to myself, Linda H. Malkas and Robert J. Hickey are co-inventors of the above-referenced patent application and co-authors of the above-referenced article.

4. Lori N. Croisetiére, Brian J. Long, Moshe Talpaz, and Lawrence Chin are named as co-authors in the above-referenced article but are not inventors of the subject matter described in the above-referenced article or in the above-referenced patent application, but were listed as co-authors of the above-referenced article in order to receive credit for having collaborated in the research program by contributing to the program.

5. Lori N. Croisetiére was a technician in the laboratory helping Pamela E. Bechtel with her project.

6. Brian J. Long was a post doc who supplied primary breast cells from Dr. Angela Brodie's laboratory.

7. Moshe Talpaz was a division chief at MD Anderson and provided sera from Chronic Myelogenous Leukemia patients under his care and from several of his residents/fellows who served as normal controls for the CML patients.

8. Lawrence Chin provided ovarian cancer tissue.

9. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issuing thereon.

DATE: 8/3/26

A handwritten signature in black ink, appearing to read 'Pamela E. Bechtel', written over a horizontal line.

Pamela E. Bechtel

EXHIBIT A

1. *An Altered Form of Proliferating Cell Nuclear Antigen in Various Cancers*, (Taken from Doctoral Dissertation of Pamela E. Bechtel entitled *Proliferating Cell Nuclear Antigen in Malignancy*, that was submitted to the Faculty of the Graduate School of the University of Maryland in 1998 and approved May 8, 1998).

Title: An Altered Form of Proliferating Cell Nuclear Antigen in Various Cancers.

Authors: Pamela E. Bechtel, Robert J. Hickey, Lori N. Croisette, Brian J. Long, Moshe Talpaz, Lawrence Chin and Linda H. Malkas

Key Words: PCNA, tumor marker, post-translational modification, epigenetic change, breast cancer, prostate cancer, malignant glioma, leukemia, cervical cancer, colon cancer

ABSTRACT

The process transforming benign cells into malignant cells involves a complex series of molecular and structural alterations which are poorly understood. One characteristic common to most malignancies is the aberrant proliferation of cells. Cellular proliferation is regulated at a number of points throughout the cell cycle through complex interaction of many proteins. Together these proteins form checkpoints that restrict the ability of cells to progress through the cell cycle. Several critically important checkpoints monitor the cells prior to the entrance into S phase. These checkpoints ultimately signal the DNA synthetic apparatus to begin DNA replication. A multiprotein DNA replication complex, DNA synthesome, has been isolated and characterized from human cells and found to be fully competent to mediate DNA replication *in vitro*. Proliferating cell nuclear antigen (PCNA) was found to be a component of the DNA synthesome. PCNA is an accessory protein for polymerase δ and is involved in DNA repair. A recent study has identified a unique form of PCNA in malignant breast cells that significantly differs in isoelectric point (pI) from the PCNA found in nonmalignant breast cells. The purpose of this study was to determine whether different types of malignant cells contain the altered form of PCNA. To examine this possibility, the DNA synthesome was isolated from malignant prostate cells (LNCaP, PC50), malignant colon cells (KGE90, KYE350, SW48), cervical cancer cells (HeLa), malignant brain cells (T98) and leukemia cells (HL60, CML, AML). The components of the synthesome were resolved by 2 dimensional gel electrophoresis (2D PAGE). Western blot analysis was performed using an antibody directed against PCNA. The results of the analysis demonstrated that the altered form of PCNA was present in all of the malignant cells examined. These findings suggest that the structural alteration of PCNA

may be involved in the development of malignancies due to the critical role PCNA has in the DNA replication and repair processes.

INTRODUCTION

Cancer is the second leading cause of death in the United States (Parker *et al.*, 1997). Lung cancer alone was responsible for more than 160,000 cancer related deaths in 1997, and it is the leading cause of cancer mortality (Parker *et al.*, 1997). Among men, prostate cancer is the second leading cause of cancer related deaths and more than 300,000 new cases were diagnosed in 1997 (Parker *et al.*, 1997). Breast cancer is the second leading cause of cancer related deaths among women and approximately 180,000 new cases of breast cancer were diagnosed in 1997 (Parker *et al.*, 1997). Colon cancer is also a leading cause of cancer deaths in men and women, with 46,000 deaths attributed to this malignancy in 1997 (Parker *et al.*, 1997; Weinberg and Strom, 1995). Leukemias and brain cancers are the most common childhood malignancies with acute lymphocytic leukemia accounting for 80% of childhood cancers (Parker *et al.*, 1997; Kumar *et al.*, 1992).

The origin and clinical manifestations of a malignancy differ for each individual cancer. Breast cancers are usually ductal or lobular in origin and generally affect post menopausal women (Dickson and Lippman, 1992). Prostate and colon cancers generally arise from benign lesions which progress into malignancies. Usually these forms of cancer affect people over the age of fifty (Isaacs, 1997; Meyers and Grizzle, 1997; Parker *et al.*, 1997; Johnson, 1995; Weinberg and Strom, 1995). Many brain tumors arise from astrocytes and are generally refractory to chemotherapy (Kordek *et al.*, 1996; Dalrymple *et al.*, 1994). Cervical cancer has been shown to be caused by human papillomavirus (HPV), and generally occurs in women over the age of thirty five (Villa, 1997; Parker *et al.*, 1997). Leukemias, in contrast to solid tumors, arise in the bone marrow and result in the circulation of immature white cells in the peripheral blood (Kumar *et al.*, 1992). Leukemia can affect people of all ages but children

with leukemias have a significantly increased survival rate compared to adults (Parker *et al.*, 1997; Kumar *et al.*, 1992).

The diagnosis of cancer at an early developmental stage is important in reducing cancer mortality. The use of mammography to detect developing breast cancers has reduced the mortality rate of the disease (Hayes, 1996). Testing for the presence of prostate specific antigen (PSA) has enabled clinicians to diagnose prostate cancer in men early in its onset (Small, 1997; Gao *et al.*, 1997). As a marker, PSA has high sensitivity and specificity and correlates with tumor burden (Pandha and Waxman, 1995; Magdelenat, 1992). However, a major limitation is the inability to distinguish between benign and malignant prostate lesions by evaluating PSA (Pandha and Waxman, 1995; Magdelenat, 1992). Routine Pap smear screens have been routinely used for the past twenty years and can be beneficial for the early detection and monitoring of cervical cancer among women. Although screening methods exist to detect certain types of malignancy the basic mechanisms for the development of cancer have not been determined.

One commonality of virtually all malignancies is the aberrant proliferation of cells. Aberrant cell proliferation is presumably due to escape from the normal controls which regulate the progression of the cell into specific stages of the cell cycle. Recently, an altered form of the protein proliferating cell nuclear antigen (PCNA) was identified from malignant human breast cells (Bechtel *et al.*, submitted). PCNA is a nuclear protein that is required for DNA synthesis and DNA repair. In this report, PCNA was analyzed from a variety of human malignancies to determine whether the unique form of PCNA was present only in malignant breast cells or whether it was present in other types of malignant cells. Several malignant human cell lines were used: LNCaP and PC50 (prostate adenocarcinoma); KGE90, KYE350 and SW48 (colon adenocarcinoma); T98

(malignant glioma); HeLa (cervical carcinoma); and HL60 (promyelogenous leukemia). PCNA was also analyzed from chronic myelogenous leukemia specimens, acute myelogenous leukemia sample and serum collected from cancer free individuals.

METHODS

Cell culture: HeLa cells were maintained in Dulbecco's Modified Eagles Medium (DMEM) with 10% bovine calf serum. HL60 and LNCaP cells were maintained in RPMI 1640 media with 10% fetal bovine serum. PC50 cells were maintained in Ham's F12K media with 7% fetal bovine serum. KGE90, KYE350 and SW48 cells were maintained in L-15 medium with 10% bovine calf serum. T98 cells were maintained in Eagles MEM with nonessential amino acids, 1.0 mM Sodium pyruvate and Earles BSS, and 10% fetal bovine serum.

DNA synthesesome isolation: The DNA synthesesome was isolated from LNCaP, PC50, KGE90, KYE350, HL60, HeLa, T98 and leukemia cell pellets according to published procedures (Coll *et al.*, 1996) with minor modifications. Briefly, cell pellets were Dounce homogenized in a buffer containing 200 mM sucrose, 50 mM Hepes, 5 mM KCl, 2 mM DTT and 0.1 mM PMSF and centrifuged at 2500 rpm for 10 min to remove cell debris and pellet the nuclei. EDTA and EGTA were added to a final concentration of 5 mM to the supernatant. One volume of nuclear extraction buffer (350 mM KCl, 50 mM Hepes, 5 mM MgCl₂, 5 mM EDTA, 5 mM EGTA, 1 mM DTT, 0.1 mM PMSF) was added to the pellet and rocked at 4°C for 2 hr. The supernatant was centrifuged at 18,000 rpm for 3 min. The supernatant was removed and centrifuged at 60,000 rpm for 22 min. The resulting supernatant was collected (S3 fraction). The nuclear pellet was centrifuged at 22,000 rpm for 3 min. The supernatant was removed and

centrifuged at 60,000 rpm for 15 min. The resulting supernatant (NE fraction) was combined with the S3 fraction, layered on top of a 2 M sucrose cushion and centrifuged overnight at 40,000 rpm. The synthesize fraction was collected for analysis.

2 Dimensional polyacrylamide gel electrophoresis (2D PAGE): DNA synthesize protein (20-40 μ g) was loaded onto the first dimension tube gel (9.2 M urea, 4% acrylamide, 2% ampholytes (pH 3-10), and 20% Triton X-100). The polypeptides were separated along a pH gradient established using 100 mM NaOH and 10 mM H₃PO₄. The tube gels were placed onto an 8% acrylamide SDS gel, and the polypeptides were resolved by molecular weight. The proteins were then transferred electrophoretically to nitrocellulose membranes.

Western blot analysis: An antibody directed against PCNA (PC 10, Oncogene Science) was used at a dilution of 1:1000. Immunodetection of PCNA was performed using a light enhanced chemiluminescence system (Amersham).

Leukemia samples: Chronic myelogenous leukemia (CML) samples were obtained from Dr. Moshe Talpaz through a collaboration with the MD Anderson Cancer Center. The acute myelogenous leukemia (AML) sample was obtained from Dr. Lynn Abruzzo through a collaboration with the Greenebaum Cancer Center.

RESULTS

PCNA in Malignant Prostate Cells

The DNA synthesize was isolated from LNCaP and PC50 prostate adenocarcinoma cell lines and the components of the synthesize were resolved by two dimensional gel electrophoresis (2D PAGE). Western blot analysis was performed using an antibody directed against PCNA to determine whether the acidic form of PCNA was present in the malignant prostate cells. The acidic form of PCNA was readily detected in both of the prostate cancer cell lines examined (Figure 1).

PCNA in Malignant Colon Cells

PCNA associated with the DNA synthesize was isolated from three colon adenocarcinoma cell lines, KGE90, KYE350 and SW48. The components of the synthesize were resolved by 2D PAGE followed by Western blot analysis using an antibody directed against PCNA. The results of this analysis indicate that the three colon cancer cell lines contained the acidic form of PCNA (Figure 2). Two of the cell lines (KGE90 and KYE350) only contained the acidic form of PCNA while the SW48 cell line contained both forms of PCNA.

PCNA in Malignant Brain and Cervical Cells

PCNA was isolated from a malignant glioma cell line (T98) and a malignant cervical cancer cell line (HeLa) to determine whether the altered form of PCNA was present. 2D PAGE and Western blot analyses were performed using a PCNA specific antibody. Figure 3A shows that the altered form of PCNA was present in HeLa cells. Figure 3B illustrates that T98 cells contained the acidic form of PCNA.

PCNA in Leukemia

Blood samples from patients with chronic myelogenous leukemia (CML) and acute myelogenous leukemia (AML), serum collected from cancer free individuals, and a human leukemia cell line (HL60) were analyzed to determine whether the acidic form of PCNA was present. The DNA synthesome was isolated from the leukemia samples and the HL60 cell line and the components resolved by 2D PAGE followed by Western blot analysis using an antibody directed against PCNA. The protein migration pattern exhibited by PCNA is shown in Figure 4. The acidic form of PCNA was identified in HL60 cells (Figure 4A), the AML sample (Figure 4B) and all of the CML samples (Figures 4C-E). The acidic form of PCNA was not detectable in the serum collected from cancer free individuals.

DISCUSSION

The diagnosis of cancer during its earliest stages of development is important in reducing cancer related mortality. Currently, a major limitation in the diagnosis of most cancers is the inability to reliably detect tumors prior to the onset of clinical manifestations. The identification of specific cellular transformation events that occur during the initial phase of tumor development may be beneficial in identifying novel tumor markers to better detect and monitor malignancies. A specific molecular alteration was recently identified in breast cancer cells which has the potential to serve as a breast cancer tumor marker (Bechtel *et al.*, submitted). To determine whether the altered form of PCNA was also present in other types of cancer cells, several different human malignancies were evaluated.

Analysis of prostate cancer cells in this study demonstrated the presence of the altered form of PCNA. Previous reports indicated that PCNA is strongly expressed in prostate cancers and also has been detected in dysplastic luminal cells of intraepithelial neoplasia, a premalignant prostate lesion (Pandha and Waxman, 1995; Magdelenat, 1992). Gao *et al.* (1997) reported that PCNA levels correlate with the clinical stage and metastatic state of prostate tumors. The researchers suggested that PCNA levels have the potential to be used as a prognostic marker for prostate cancer (Gao *et al.*, 1997).

The colon cancer cell lines examined in this study also contained the altered form of PCNA. Studies have shown that PCNA labeling index is correlated with tritiated thymidine uptake in assessing the proliferative activity of colon tumors (Bleiberg *et al.*, 1993). Neoptolemos *et al.* (1995) demonstrated that PCNA levels used in conjunction with the Duke's classification system (the classification system for colon tumors based on histological examination) improved the prediction of survival for colon cancer patients. Mitchell *et al.* (1997) demonstrated that increased levels of PCNA are found in primary colon tumors and in resulting lung metastases. It has been shown that PCNA levels increase during the progression of benign lesions to malignancy and that increased levels are associated with the recurrence of adenomas (Shpitz *et al.*, 1997; Paspatis *et al.*, 1994; Risio *et al.*, 1993). Risio *et al.* (1993) hypothesized that the increase in the number of PCNA labeled cells is related to hyperproliferation as well as to the neoplastic deregulation of PCNA synthesis in the development of colon cancer.

Malignant gliomas are composed of heterogeneous subpopulations of cells with different cytogenetic compositions making it difficult to identify useful prognostic markers (Dalrymple *et al.*, 1995). Previous studies demonstrated that PCNA labeling index correlates with the histological grade of the brain

malignancy. In the majority of benign astrocytomas, the PCNA labeling index is low (Kordek *et al.*, 1996). Kordek *et al.* (1996) also have hypothesized that malignant gliomas contain two forms of PCNA, one of which does not have a role in DNA synthesis. The results of the present study have indicated that malignant glioma cells contain the altered form of PCNA. The functional role of the altered form of PCNA needs to be examined in greater detail to determine whether it has a role in the development and progression of malignant gliomas.

The role of PCNA in the development and progression of CML is not well characterized. CML is a biphasic disease characterized by an early chronic phase followed by a blast phase (Zaccaria *et al.*, 1995). Takasaki *et al.* (1984b) demonstrated a correlation between the number of leukocytes expressing PCNA and the percent of blast cells in blood during the blast phase of CML. These investigators also identified the presence of non-blast cells which were positive for PCNA in the peripheral blood during the blast phase of CML. This result differs from the observation that the non-blast cells were negative for PCNA in the chronic phase (Takasaki *et al.*, 1984). The PCNA labeling index for CML cells is not significantly different from normal bone marrow cells (Thiele *et al.*, 1993). However, in the chronic myeloid proliferative disorder osteomyelofibrosis, there is a significant increase in the PCNA labeling index (Thiele *et al.*, 1994). Interferon treatment resulted in decreased PCNA labeling. In the present study, the results demonstrated that the leukemia samples examined contain the altered form of PCNA, while samples collected from cancer free individuals did not contain the altered form of PCNA.

Analysis of HeLa cells in the present study revealed that these cervical cancer cells contain the altered form of PCNA. Cardillo *et al.* (1993) found that PCNA levels were increased in all cases of invasive squamous carcinoma, cervical adenoma, cervical carcinoma in situ (CIS) and cervical intraepithelial

neoplasia (CIN) III examined. Smela *et al.* (1984) demonstrated a significant correlation between PCNA index and the severity of cervical dysplasia by determining the PCNA levels in CIN I, CIN II and CIN III lesions. Other studies found that PCNA index was increased in premalignant and malignant cervical lesions compared to nonmalignant tissue (Steinbeck *et al.*, 1995; Raju *et al.*, 1994; Shurbaji, 1993). Kobayski *et al.* (1994) concluded that the increase in PCNA labeling index suggests that there is considerable alteration of biologic activity, including genetic mutations, during the progression of severe cervical dysplasia to CIS.

This study examined several human malignancies for the presence of the acidic form of PCNA. The malignancies examined in this study have few common characteristics or clinical manifestations. Previously, an altered form of PCNA was identified in malignant breast cells. It was demonstrated that the unique form of PCNA was not the result of growth stimulation or genetic mutation (Bechtel *et al.*, submitted). Further research indicated that differential post-translational modification by poly(ADP-ribosylation) was at least partially responsible for the presence of the altered form of the protein (Bechtel *et al.*, submitted). This report identified the acidic form of PCNA in leukemia, prostate, cervical, colon and brain cancers. These data demonstrated a simple molecular alteration in the PCNA polypeptide is a common feature between these diverse types of cancer. These findings suggest that the acidic form of PCNA may have an underlying role in the development of different types of cancer and that these cancers may develop as a consequence of a global alteration in the manner in which specific cellular proteins are post-translational modified.

REFERENCES

Bechtel, P.E., Hickey, R.J., Schnaper, L., Sekowski, J.W., Long, B.J., Freund, R., Lui, N., Rodriguez-Valenzuela, C. and Malkas, L.H.: A unique form of proliferating cell nuclear antigen (PCNA) in breast cancer cells. (submitted).

Bleiberg, H., Morret, M. and Galand, P. (1993): Correlation between [3H] thymidine and proliferating cell nuclear antigen (PCNA/ cyclin) indices in archival, formaldehyde-fixed human colorectal tissues. *Europ. J. Cancer* **29A**(3): 400-403.

Cardillo, M.R., Stamp, G.W., Pignatelli, M.N. and Lalani, E.N. (1993): Immunohistochemical analysis of p53 oncoprotein and proliferating cell nuclear antigen (PCNA) in the cervix uteri. *European Journal of Gynaecological Oncology* **14**(6): 484-490.

Coll, J.M., Sekowski, J.W., Hickey, R.J., Schnaper, L., Yue, W.Y., Brodie, A.M.H., Uitto, L., Syvaaja, J.E. and Malkas, L.H. (1996): The Human Breast Cell DNA Synthesome: Its Purification from Tumor Tissue and Cell Culture. *Oncology Research* **8** (10/11): 435-447.

Dalrymple, S.J., Parisi, J.E., Roche, P.C., Ziesmer, S.C., Scheithauer, B.W. and Kelly, P.J. (1994): Changes in proliferating cell nuclear antigen expression in glioblastoma multiforme cells along a stereotactic biopsy trajectory. *Neurosurgery* **35**(6): 1036-1044.

Dickson, R.B. and Lippman, M.E.: Molecular basis of breast cancer. In. Molecular Basis of Cancer..

Fujiwaki, R., Takahashi, K. and Kitao, M. (1997): Decrease in tumor volume and histologic response to intra-arterial neoadjuvant chemotherapy in patients with cervical and endometrial adenocarcinoma. *Gynecologic Oncology* **65**(2): 258-264.

Gao, X., Porter, A.T., Grignon, D.J., Pontes, J.E. and Honn, K.V. (1997): Diagnostic and prognostic markers for human prostate cancer. *The Prostate* **31**: 264-281.

Hayes, D.F. (1996): Serum (Circulating) tumor markers for breast cancer. *Recent Results in Cancer Research* **140**: 101-113.

Henson, D.E., Fielding, L.P., Grignon, D.J., Page, D.L., Hammond, M.E., Nash, G., Pettigrew, N.M., Gorstein, F. and Hutter, R.V.P. (1994): College of American Pathologists Conference XXVI on clinical relevance of prognostic markers in solid tumors. *Arch. Pathol. Lab. Med.* **119**: 1109-1112.

Isaacs, J.T. (1997): Molecular markers for prostate cancer metastasis. *Amer. J. Path.* **150** (5): 1511-1521.

Johnson, I.T. (1995): Butyrate and markers of neoplastic change in the colon. *Eur. J. Cancer Preven.* **4**: 365-371.

Kobayashi, I., Matsuo, K., Ishibashi, Y., Kanda, S. and Sakai, H. (1994): The proliferative activity in dysplasia and carcinoma in situ of the uterine cervix analyzed by proliferating cell nuclear antigen immunostaining and silver binding

to the argyrophillic nucleolar organizer region staining. *Human Pathology* **25**: 964.

Kordek, R., Beirnat, W., Alwasiak, J. and Liberski, P.P. (1996): Proliferating cell nuclear antigen (PCNA) and Ki-67 immunopositivity in human astrocytic tumors. *Acta Neurochirurgica* **138** (5): 509-512.

Kumar, V., Cotron, R.S. and Robbins, S.L. (Eds.): Basic Pathology. W.B. Saunders Co. Philadelphia, PA.

Magdelenat, H. (1992): Tumour markers in oncology: past, present and future. *Journal of Immunological Methods* **150**: 133-143.

Meyers, R.B. and Grizzle, W.E. (1997): Changes in biomarker expression in the development of prostate adenocarcinoma. *Biotechnic and Histochemistry*. **72**(2): 86-95.

Mitchell, B.S., Horny, H.P. and Schumacher, U.K. (1997): Immunophenotyping of human HT29 colon cell primary tumours and their metastases in severe combined immunodeficient mice. *Histochemical Journal* **29**(5): 393-399.

Nakai, H. and Misawa, S. (1995): Chromosome 17 abnormalities and inactivation of the p53 gene in chronic myeloid leukemia and their prognostic state. *Leukemia and Lymphoma* **19**: 213-221.

Neoptolemos, J.P., Oates, G.D., Newbold, G.D., Robson, A.M., McConkey, C. and Powell, J. (1995): Cyclin/proliferating cell nuclear antigen

immunohistochemistry does not improve the prognostic power of Dukes' or Jass' classifications for colorectal cancer. *British J. Sur.* **82**(2): 184-187.

Oka, K., Hoshi, T. and Arai, T. (1992): Prognostic significance of the PC10 index as a prospective assay for cervical cancer treated with radiation therapy alone. *Cancer* **70**(6): 1545-1550.

Pandha, H.S. and Waxman, J. (1995): Tumour markers. *Q.J. Med.* **88**: 233-241.

Parker, S.L., Tong, T., Bolden, S. and Wingo, P.A. (1997): Cancer statistics 1997. *CA Journal for Clinicians* **47**(1): 5-27.

Paspatis, G.A., Karamanolis, Vasilakaki, T., Zizi, A., Xourgias, V., Elemenoglou, I. Hadziyannis, S.J. (1995): Proliferative activity in colonic adenomas as a predictor of metachronous adenomas as assessed by proliferating cell nuclear antigen immunohistochemistry. *Amer. J. Gastroenterology* **90**(4): 597-602.

Raju, G.C. (1994): Expression of the proliferating cell nuclear antigen in cervical neoplasia. *International Journal of Gynecological Pathology*. **13**(4): 337-3341.

Risio, M., Candelaresi, G. and Rossini, F.P. (1993): Bromodeoxyuridine uptake and proliferating cell nuclear antigen expression throughout the colorectal tumor sequence. *Cancer Epidemiology, Biomarkers & Prevention* **2**(4): 363-367.

Shpitz, B., Bomstein, Y., Mekori, Y., Cohen, R., Kaufman, Z., Grankin, M. and Bernheim, J. (1997): Proliferating cell nuclear antigen as a marker of cell kinetics

in aberrant crypt foci, hyperplastic polyps, adenomas, and adenocarcinomas of the human colon. *Amer. J. Sur.* **174**(4): 425-30.

Shurbaji, M.S., Brooks, S.K. and Thurmond, T.S. (1993): Proliferating cell nuclear antigen immunoreactivity in cervical intraepithelial neoplasia and benign cervical epithelium. *Amer. J. Clin. Path.* **100**(1): 22-26.

Small, E.J. (1997): Prostate cancer. *Current Opinion in Oncology* **9**: 277-286.

Smela, M., Chosia, M. and Domagala, W. (1996): Proliferating cell nuclear antigen (PCNA) expression in cervical intraepithelial neoplasia (CIN). An immunohistochemical study. *Polish J. Path.* **47**(4): 171-174.

Steinbeck, R.G., Heselmeyer, K.M., Moberger, H.B. and Auer, G.U. (1995): The relationship between proliferating cell nuclear antigen (PCNA) nuclear DNA content and mutant p53 during the genesis of cervical carcinoma. *Acta Oncologica* **34**(2): 171-176.

Takasaki, Y., Robinson, W.A. Tan, E.M. (1984): Proliferating cell nuclear antigen in blast crisis cells of patients with chronic myeloid leukemia. *J. Natl. Cancer Inst.* **73**(3): 655-661.

Thiele, J., Hoefer, M., Kvasnicka, H.M., Bertsch, H.P., Zankovitch, R. and Fischer, R. (1993): Erythropoiesis in CML-immunomorphometric quantification, PCNA-reactivity, and influence on survival. *Hematologic Path.* **7**(4): 239-249.

Thiele, J., Bertsch, H.P., Kracht, L.W., Anwander, T., Zimmer, J.D., Kreipe, H. and Fischer, R. (1994): Ki-67 and PCNA expression in erythroid precursors and megakaryocytes—a comparative study on proliferative and endoreduplicative activity in reactive and neoplastic bone marrow lesions. *J. Path.* **173**(1): 5-12.

Villa, L.L. (1997): Human papillomaviruses and cervical cancer. *Advances in Cancer Research* **71**: 321-341.

Weinberg, D.S. and Strom, B.L. (1995): Screening for colon cancer: A review of current and future strategies. *Seminars in Oncology*. **22** (5): 433-447.

Zaccaria, A., Martinelli, G., Testoni, N., Zuffa, E., Farabegoli, P., Russo, D., Guerrasio, A. and Tura, S. (1995): Does the type of BCR/ABL junction predict the survival of patients with Ph¹-positive chronic myelogenous leukemia? *Leukemia and Lymphoma* **16**: 231-236.

Zdziarska, B. and Nowacki, P. (1996): Leukemic cells growth fraction in the central nervous system in the blastic phase of chronic myelogenous leukemia. *Acta Haematologica Polonica* **27**: 43-48.

Zusman, I. (1995): Variability of neoplastic parameters in colon tumours and its significance in diagnostic practice. *Biol. Rev.* **70**: 107-160.

FIGURE LEGENDS

Figure 1: The protein migration pattern of PCNA from malignant prostate cells. The DNA synthesize was isolated from LNCaP and PC50 prostate cancer cells. The components of the synthesize were resolved by 2D PAGE, and subjected to Western blot analyses using an antibody directed against PCNA. The electrophoretic mobility of PCNA is shown for (A) LNCaP cells and (B) PC50 cells. The arrow indicates the position of the acidic form of PCNA.

Figure 2: The protein migration pattern of PCNA from malignant colon cells. The DNA synthesize was isolated from KGE90, KYE350 and SW48 colon cancer cells. The components of the synthesize were resolved by 2D PAGE and subjected to Western blot analyses using an antibody directed against PCNA. The electrophoretic mobility for PCNA is shown for (A) KGE90 cells, (B) KYE350 cells and (C) SW48 cells. The arrow indicates the position of the acidic form of PCNA.

Figure 3: The protein migration pattern of PCNA from malignant brain and cervical cells. The DNA synthesize was isolated from malignant glioma (T98) and cervical (HeLa) cells. The components of the synthesize were resolved by 2D PAGE and subjected to Western blot analyses using an antibody directed against PCNA. The electrophoretic mobility for PCNA is shown for (A) HeLa cells and (B) T98 cells. The arrow indicates the position of the acidic form of PCNA.

Figure 4: The protein migration pattern of PCNA from leukemia cells. The DNA synthesize was isolated from HL60 cells, CML samples, an AML sample

and serum collected from cancer free individuals. The components of the synthesize were resolved by 2D PAGE and subjected to Western blot analyses using an antibody directed against PCNA. The electrophoretic mobility for PCNA is shown for (A) HL60 cells, (B) AML cells and (C-E) CML cells. The arrow indicates the position of the acidic form of PCNA.

Figure 1

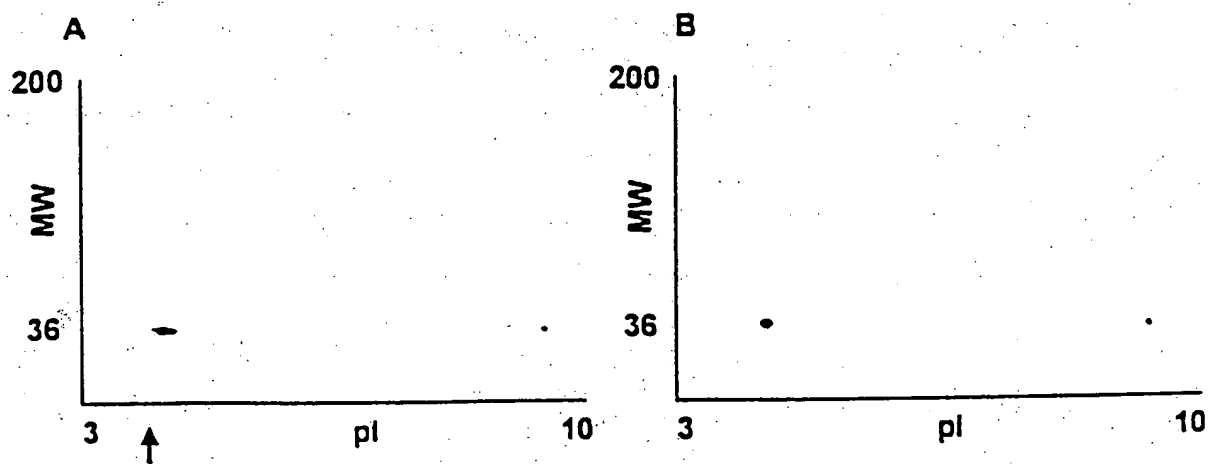


Figure 2

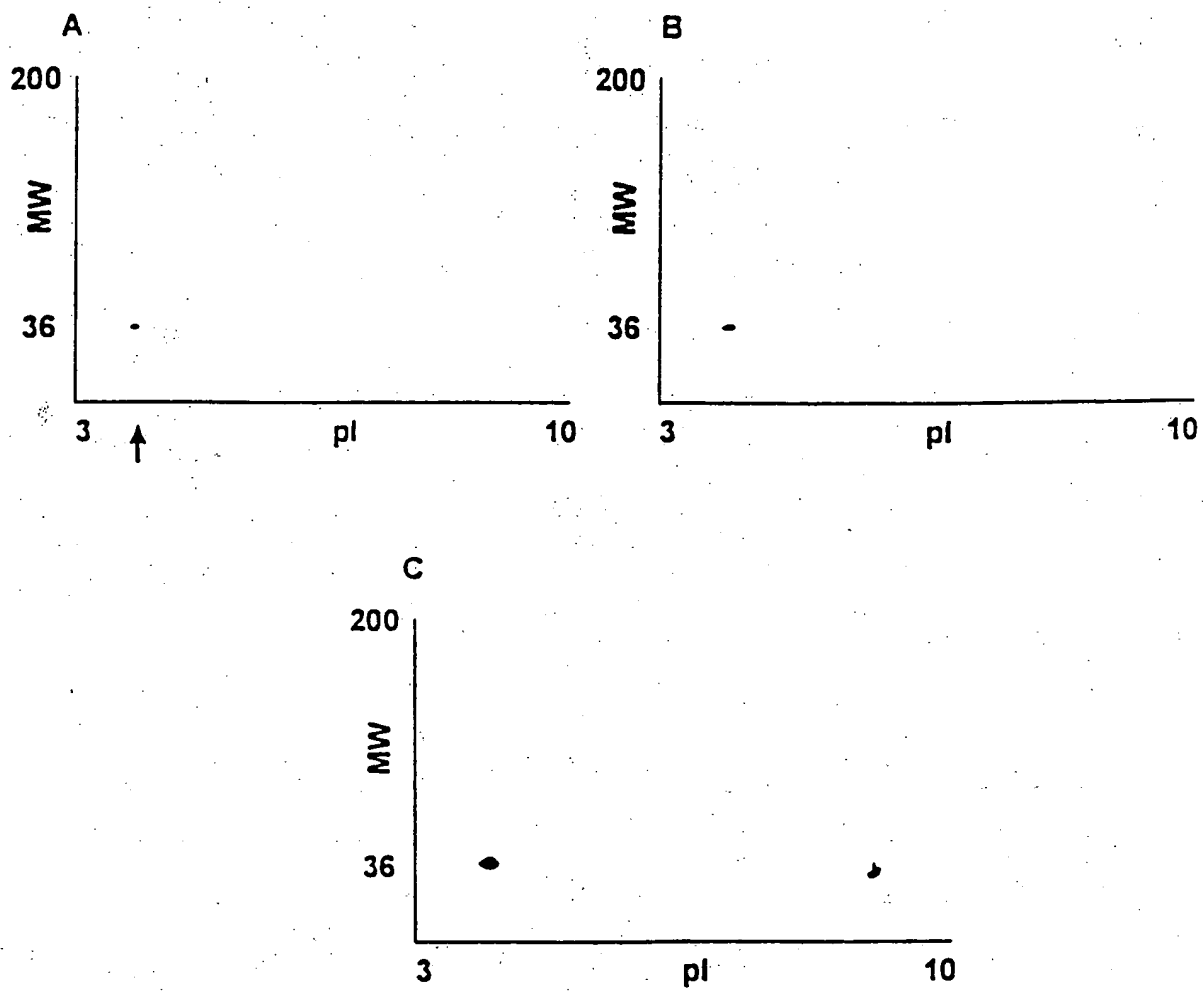


Figure 3

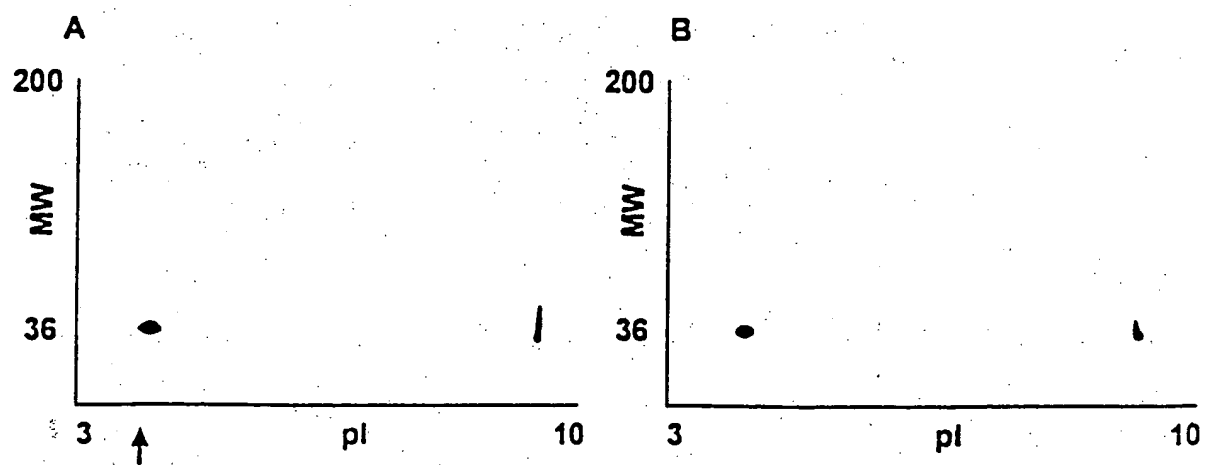


Figure 4

